

104

Final Technical Report

SIMULATED WEIGHTLESSNESS IN FISH AND
NEUROPHYSIOLOGICAL STUDIES ON MEMORY STORAGE

by

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TITLE: Simulated Weightlessness in Fish and Neurophysiological Studies on Memory Storage.

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1.) Simulated Weightlessness in Fish:

a.) The gravity reference response:

Blind unrestrained goldfish with intact otolith system were used in these experiments. Fish proved to be very suitable for these studies because they readily respond to changes of the gravitoinertial field which represents a stimuli for vestibular reflexes. The behavioral responses of the fish in the tank were recorded by high speed cinematography and then studied by single frame analysis of the film.

Blind fish when accelerated forward in a sportscar responded by assuming a forward tilted diving position in the tank and a backward tilted climbing position when accelerated backward. The actual tilt angle was greater than the theoretical tilt angle, i.e. the angle between the resultant of the gravitational and inertial force and the longitudinal axis of the fish. This response was called "gravity reference response"^{1,2} and was not measurably influenced by any concomitant signals from the system of the semicircular canals. In addition to the gravity reference response, a forward "darting" response was observed

when the acceleration was increased suddenly (high jerk load, g/sec.).

The gravity reference response in fish seems to be relevant to some illusory sensations man experiences during linear acceleration. Jet pilots when catapulted from aircraft report feeling tilted backward. Human subjects on centrifuges experience an apparent displacement of fixed targets in their visual field (oculogravic illusion).

b.) The low G diving response³:

Through use of aircraft blind goldfish were exposed to different G loads and weightlessness. The responses of the fish were recorded together with the accelerations to which they were subjected. A movie camera and oscillograph were used to record the data. A small jet transport aircraft was used for our experiments which allowed periods of weightlessness of up to 30 seconds in parabolic flights. In some experiments a light propeller driven aircraft was used (Cessna 172) which allowed weightlessness for 5-8 seconds. The portions of the parabolic flight trajectory are referred to, in sequence, as: pull up, or high G load; push over, or less than 1 g load; parabola, or 0 g load; and pull out, or high G load. Acceleration indicators were provided in the cockpit to allow the pilot to perform the prescribed flight patterns. The pilot usually controlled the G load in the Z-axis with the aircraft elevator control while the co-pilot controlled the G load in the X-axis by controlling the engine's power with the throttles. In later flights the cockpit acceleration indicators were servo-motor driven, as were the acceleration indicators in the experiment display³.

In addition to parabolas of maximum duration various other and more complex flight patterns were executed, e.g., parabolas of a low

magnitude positive or negative G load, parabolas in which the G load was varied between low positive and negative G load magnitudes or 0 g and a low positive G load magnitude, parabolas in which the aircraft vertical G load was maintained at zero while longitudinal accelerations were applied through manipulation of the aircraft engine's power with the throttles, "roller coaster" maneuvers in which the aircraft's vertical G load varied between magnitudes of 0.2 g and 2 g and pull ups and push overs at prescribed high or low jerks such as approximately 5 g/second or 0.15 g/second.

c.) Diving response^{3,4}:

A diving response by the fish occurred between one and 0 g regardless of whether the G force was decreasing or increasing. This response occurred during the push over and pull out from the weightless parabola. The fish actively assumed a nose down or forward tilted position. The angle of the dive became steeper as the G load decreased toward zero.

During the push over the fish, in most cases, started a dive from a horizontal position. During the pull out when the gravity force returned after a period of weightlessness, the fish went into a dive regardless of which random position he was in. Single frame analyses of movies of the experiment were performed to further evaluate the diving response. High G loads of approximately 3 g obtained during the pull ups, and the rapid decrease of the high G loads toward 1 g did not have any noticeable effect on the fish. The forward tilt commenced when the G load decreased below the level of 1 g, after an average latency period of 200-300 msec. When the fish reached the bottom of the tank they usually stayed there in a forward tilted position without measurable

adaptation. The fish displayed hysteresis on pull out from weightlessness as they did not return to the original horizontal position immediately when 1 g was again reached, but only after a delay of up to 3 seconds.

d.) Looping response³:

The looping response was the most spectacular fish response uncovered by these studies. It appeared in the proximity of weightlessness. The fish is in a near-vertical dive (diving response during push over to 0 g) before commencing the looping response². To start the looping response the fish continues nosing over past a vertical attitude into an inverted attitude and continues to loop in this manner, without measurable adaptation, throughout the duration of weightlessness, i.e., up to 30 seconds in our tests. The average angular speed of rotation remained nearly constant in many cases but in some cases this speed increased with increased exposure to weightlessness. A positive feedback due to the centrifugation of the otoliths could be the cause of increased angular speed.

At the end of the weightless parabola, with the initiation of the pull out when the G force sets in, the fish quickly assumes a belly down attitude and displays diving response. If the fish is in an inverted attitude at the bottom of the loop, he normally rolls about its longitudinal axis to a level attitude and then commences a near-vertical dive. In two experiments, in which a seeing fish was flown simultaneously with a blind fish, only the blind fish showed the looping response during 0 g.

e.) Inverted diving response and response to oscillation about a 0 g baseline³:

By increasing the angular velocity within the vertical plane in which the parabola is flown, the centrifugal force vector can be increased so that the resulting gravito-inertial vector becomes negative and the G force vector is directed toward the top of the fish tank. This condition was identified by negative vertical (Z-axis) G value and was marked by air bubbles descending to the bottom of the fish tank. The fish in all cases recognized this condition very quickly. The threshold magnitude was less than 0.1 g. The fish response was to roll to a belly-up attitude and then perform an "inverted dive" toward the top of the tank. Thus, the fish actively swam obliquely upward to the top of the tank and stayed there in a belly-up tilted position for the total duration of the negative G load. At the end of the parabolic trajectory when the pull out began and the G load became positive again, the fish rolled belly-down and dove to the bottom of the tank in a typical diving response. The shift from the inverted to the normal diving response appeared to occur approximately simultaneously with the shift of the air bubbles from the bottom to the top of the tank at the beginning of the pull out.

In five experiments during the weightless parabola the aircraft was not consistently flown at 0 g, but the control columns were gently moved forward and backward so that a G load oscillating between ± 0.1 g was generated. This minor oscillation about 0 g caused a dramatic response from all fish. Consistently, a deviation from 0 g to slightly negative G values caused the fish to turn belly-up and swim straight up to the top of the tank. A deviation of the G load toward slightly positive G values caused the fish to point straight

down and quickly dive to the bottom of the tank. The fish movement always opposed the movement of any air bubbles present in the tank; if the air bubbles moved down, the fish moved up and vice versa. This cycle could be repeated 3-4 times during a parabola. The threshold for this "turnaround response" was hardly measurable, but in any case was smaller than 0.1 g magnitude. In summary, a shift in the direction of a low magnitude acceleration in weightlessness causes a rapid 180° turn in the blind fish, while a shift in the direction of an applied acceleration in the Earth's gravitational field would not be significant because of a higher acceleration magnitude threshold than during the 0 g condition. This increased responsiveness seems to be explained best by a combination of directional sensitivity (which should give the most obvious response to a vectorial change of 180°) with a Weber-Fechner relationship of increased receptor sensitivity at diminished levels of background stimulation.

f.) Significance of these findings in relation to man^{5,6,7}:

The significance of this observation for manned space flight would be that minor linear accelerations, as caused by locomotion or head movements, could be expected to lead to abnormally strong vestibular reactions. Even if any reflectory movements are voluntarily suppressed, a continued exposure to such strong stimuli would cause subjective sensations. Vestibular overstimulation, especially when combined with a conflict between visual and vestibular information, have been suggested as important causes of motion sickness. Our observations that seeing fish do not, or to a much lesser degree, respond to lowered G force as do blind fish suggest the presence of a

conflict between visual and vestibular information. Spatial illusions, inverted body position sensations as well as the nauseating symptoms the astronauts and cosmonauts experienced in space flights, may well be caused by accelerations due to head movements and locomotion during space flight and would strongly support this view. With the current plans and future objectives for space flights of increasing duration, continued research toward a better understanding of the vestibular mechanism during the state of weightlessness is required.

2.) Neurophysiological Studies of the Statocyst Nerve of the Gastropod Mollusc Pleurobranchaea Californica

a.) Activity recorded from the statocyst nerve of Pleurobranchaea Californica during rotation and at different tilts⁸:

For this study done in collaboration with Dr. J. Wood, we used the relatively simple system of equilibrium of a mollusc in order to better understand how the more complex otolith systems of vertebrates operates.

In Pleurobranchaea there are 13 large receptor cells in each statocyst and 13 axons in each statocyst nerve. Few statoconia remain in histological preparations, but under the dissecting microscope the statolith appears spherical with a diameter of about 150 μ within the 200 μ diameter statocyst. The electrical spike activity of single fibers of the statocyst nerve was recorded by a suction electrode at different degrees of tilt of the statocyst⁸. Most records were analyzed on a PDP-8 computer. Subsequent data reduction employed an IBM 360 computer. The number of firings of each individual unit (recognized by amplitude histogram) was plotted against the time of firing, and scatter plots of

interspike interval vs. time of occurrence were generated. Phase and latency relationships between different units active at the same position were also calculated.

Recordings from the statocyst nerve showed several units spontaneously active at any one position. Continuous rotation demonstrated that each unit responds with maximum frequency soon after rotation into its active range. After continuous rotation past the position of maximum response, immediate rotation back to the position of maximum response elicits less activity than was initially elicited. During rotation in small increments, each unit responded with increased frequency over approximately the same range of angular positions regardless of the direction of rotation about a given axis, but maximum numbers of impulses were recorded in particular positions. These positions differed when the preparation was rotated in opposite directions. Regardless of the direction of rotation, the maximum average frequency occurred close to the position at which the unit first became excited and average frequency declined beyond this point. Response to rotation about the animal's longitudinal axis was recorded from a single unit in the left statocyst nerve. This preparation was rotated incrementally through 360° clockwise (toward right side down) and then counter-clockwise through 360° . Maximum impulse frequencies over the 2-min period were recorded at orientations which differed by 80° .

Rotations through repeated sequences of positions yielded reproducible changes in the average frequency of single units. As in the case of rotations through 360° , the initial frequency at a particular position depended upon the direction from which the position was

approached. The firing level finally approached a steady rate, which was characteristic of the position and the unit. In another experiment the average impulse frequency over the 2-min period following rotation to the horizontal was lower when the direction of approach was clockwise than when the direction of approach was counter-clockwise. Counter-clockwise rotation to the horizontal caused an immediate increase in average frequency; while clockwise rotation to the horizontal resulted in an immediate decrease in average frequency. During the 2-min period following rotation to the horizontal, the higher frequency (following counter-clockwise rotation) decreased while the lower frequency (following clockwise rotation) increased. Similar changes in frequency with time were seen after rotating the preparation from the horizontal. Clockwise rotation to 15° right side down caused a decrease in frequency which then increased with time. Data from a different experiment using smaller increments of rotation were similar to the data described above. Tonic activity continues indefinitely, but adaptation is not a simple exponential decay of frequency over time.

No temporal relationships indicating synaptic interactions between receptor cells were found under the conditions of these experiments. Compound action potentials were occasionally seen. They did not occur regularly but occurred when two units, firing at different frequencies happened to fire simultaneously. From the results it follows that each unit in the statocyst nerve of *Pleurobranchaea* responds over a limited range of positions. The receptor adaptation is slow. Each position in the active range of a unit is coded by the same steady-state

firing rate. Initial frequencies upon reaching a particular position differ, depending upon the direction of approach.

3.) Neurophysiological Studies on Memory Storage⁹:

Information storage was investigated in relatively simple neuronal networks of the mollusc *Aplysia*. In spite of extensive studies by many groups within the last decade, there are very few electrophysiological data available to support synaptic plasticity as a basis for the connectivity-hypothesis of memory. We tried to test a different hypothesis which assumes that information is stored in the form of altered parameters of self excitation of individual neurons. In order to be able to study autorhythmicity of single neurons undisturbed by any synaptical, apholactical or neurosecretory influence from surrounding neurons, we developed a method to completely isolate live nerve cells from *Aplysia*-Ganglia.³

Ganglia of *Aplysia Californica* were dissected from the animals and immersed in 0.25% trypsin dissolved in artificial seawater for 3 hrs at 35°C. After being washed in seawater for 10 min., the ganglia were immersed in a plastic recording chamber filled with seawater. The connective tissue capsules of the ganglia, already softened by the trypsin, could be stripped off easily. Careful teasing with micro-dissecting pins released many isolated neurons with axons 100 μ to 800 μ long. The diameters of the somata ranged from 50 μ to 800 μ . Glia could be completely stripped from the neurons. The penetration of isolated neurons with microelectrodes was even easier than in intact or opened ganglia. The yield of viable cells for recording is at least 50% of the total number of isolated neurons.

Control studies were made on the effects of trypsin on the bioelectric activity of cells within the intact ganglion. Trypsin impaired neuronal activity only temporarily. One hour after immersion in trypsin, the cells were depolarized by 10-15 mV and only very small spontaneous spikes appeared infrequently. After 3 h of trypsin treatment, all spontaneous activity had ceased and the membrane potential was reduced to 20-45 mV.

During rinsing in seawater, the membrane potential gradually returned to the control level; spontaneous spikes reappeared and progressively increased in amplitude until normal spike size (both peak depolarization and after-spike hyperpolarization) was reached.

The method of recording from isolated neurons that is described here presents an interesting possibility. (1) All possible synaptic interactions in the neuropil and on the axonal stem are completely eliminated. The completely isolated neuron is not subject to the effects of any extracellular electric fields in the surrounding tissue, although the possible influence of such fields in other preparations has been suggested. Thus isolated neurons provide an ideal preparation for the study of membrane dynamics, oscillatory properties, rhythmicity and the origin of pacemaker potentials. (2) The influence of surrounding glial tissue can be excluded. These recordings from completely stripped nerve cells show that *Aplysia* cells are capable of auto-rhythmicity without glia-neuron interaction. If there are any reminders of glia membranes left in somatic indentations, they are completely broken off the glia cells. (3) The effects of various chemical agents and ionic compositions of the bath solution can be

studied directly, without any interference or delay by the surrounding tissue, which can not fully be avoided even in surgically opened ganglia.

Intracellular electrical stimulation of such isolated Aplysia neurons has shown that a manipulation of autotonomous rhythmicity is possible. This represents a form of information storage.

In order to explain better the internal mechanism underlying endogenous nerve cells rhythmicity and its changes by electrical stimulation, we began to study the biochemical systems involved. We found that glycolysis blocking reagents, such as sodium iodoacetate and heavy water slow down or block autorhythmicity without altering markedly the resting potential of the membrane. Addition of glucose to the environment of a nerve cell which is blocked by such agents has a remedial effect on autorhythmicity. We therefore can conclude that glycolysis and oxydative phosphorylation are involved in the inherent rhythmicity of Aplysia neurons. Similar studies including a voltage clamp are in progress.

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